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SURVIVAL OF PATHOGENIC STAPHYLOCOCCI AND STREPTOCOCCI IN THE AIR AND ON OBJECTS OF THE EXPERIMENTAL UNIT

/Following is the translation of an article by V. A. Yaroshenko, Ukrainian Institute of Communal Hygiene, published in the Russian-language periodical Vop. Sanit. Bakt. Virus., 1965, pages 84--96./

(Experimental Investigations)

The role of factors of the surrounding medium, including air and objects of the working unit, in the spreading of pathogenic microbes and viruses has been established both experimentally and by clinico-epidemiological observations. Therefore the clearing up of the details of the aerial method of spreading pathogenic microbes among people creates the prerequisite for an active attack on this process, and by this promotes the development of measures for preventing the development and spreading of airborne infections. The danger of a bacterial (viral) aerosol depends not only in its stability, but also the capacity of the pathogenic microorganisms to preserve their viability and virulence while existing in the air. It is necessary to note, however, that the duration of survival and preservation of biological activity (virulence) by pathogenic microbes, including hemolytic staphylococci and streptococci, in the air and on objects of the working unit have still been studied insufficiently.

The first works on the study of survival of microorganisms which are suspended in the air were carried out in the thirties of the current century. Having sprayed broth cultures of pneumococci and hemolytic and viridans streptococci, and also the diphtheria bacillus in an air medium, W. Wells and W. Stone (1934) established that these microorganisms remain viable in the air for 48 hours.

According to the findings of S. S. Rechmenskiy (1944), beta-hemolytic streptococcus, when sprayed in the air of an isolation room at a temperature of 16-17° and humidity of 60-65%, preserves its viability in the aerosol for 20 hours. As established by the author, the viability and virulence (for white mice) of this microorganism, which had settled out from the aerosol onto glass partitions in the room, were preserved for 5 days. In the experiments of V. I. Vashkov and Ye. K. Serebryakovaya (1954) hemolytic streptococcus, suspended in the air of an experimental room, was detected 19 hours after a suspension of it was sprayed.

G. Schechmeister and L. Goldberg (1950), in studying the stability of Streptococcus zooepidemicus in air at a temperature of 18-20° and relative humidity of 38-42%, established that their survival in an aerosol depends on the nature of the liquid in which the microbial suspension being sprayed was prepared. The longest survival rate was observed in an aerosol from a suspension on tryptosophosphate broth with 10% bovine serum. W. Wells (1955), while observing viability of various microbes which were sprayed in the air at room temperature and 70% relative humidity, showed that staphylococcus is quite stable in an aerosol state. It was detected in the air on the 6th day after the experiment was set up.

V. V. Vlodavets (1956), while studying the colloidal-chemical properties of an aerosol of Staphylococcus albus, detected that at high indices of humidity (79-91%) the concentration of microbes in the dust-borne and droplet phases decreases rapidly. At a low humidity (23-35%) staphylococcus is preserved longer in the air in both phases of the aerosol. L. P. Perlina (1955) studied the survival of virulent staphylococcus in the dust-borne phase of an aerosol under the influence of natural factors of the external medium. The experiments were set up on test objects (wool and cotton) which were sown with a culture of staphylococcus, both protected by protein and also devoid of this protection. The author showed that staphylococci, protected by a protein membrane, in the dust-borne phase in diffuse light survived from 194 to 216 days and preserved their virulence for 129--170 days. If the same staphylococci were deprived of the protein protection, then in the dust-borne phase they remained viable for several days.

It was established in the observations of M. A. Zlatoust (1957) that Staphylococcus aureus, sown on surrounding objects (fragments of cotton tissue) in the form of the droplet phase of an aerosol, under conditions of darkness at room temperature preserves its viability and biological properties for 84 days. Following the admission of daylight in the room situation the staphylococci on the same objects were preserved for 42 days, but losses of their biological properties set in in the 3rd week.

It can be seen from the cited literature review that data on the influence of temperature and relative humidity, and also other natural factors of the external medium, on the survival of pathogenic staphylococci and streptococci in the air and on surrounding objects are very limited. Moreover the results of observations conducted by different investigators on the periods of survival of these microorganisms in the external environment are quite contradictory.

The objects of our investigations were cultures of hemolytic staphylococcus (7 strains) and hemolytic streptococcus (4 strains) which were freshly isolated from patients. All the strains of staphylococcus possessed well expressed hemolytic properties, caused the coagulation of citrate plasma of rabbit blood in 4-6 hours, and

caused the death of white mice in 72 hours following intraperitoneal administration. Hemolytic streptococci were characterized by typical morphological and cultural properties, and also virulence for white mice.

The tests were set up in an hermetic chamber with a capacity of 160 liters at temperature ranges which are encountered under natural conditions: from 14 to 29° and a relative humidity from 45 to 87%.

For obtaining the droplet phase of the aerosol we used suspensions of 24-hour cultures of the microorganisms being tested. Aerosols were created with the help of an electric inhaler under the action of a compressor. The inhaler made it possible to obtain drops with a diameter of 2-3 microns. The process of spraying the suspension of microbes lasted for several seconds. As liquids for suspending the 24-hour cultures of microbes we used: distilled water, meat-peptone broth, blood serum, physiological solution of sodium chloride and saliva.

For creating the dust-borne phase of the aerosol we used the dust from an inhabited accommodation. The dust, which was collected with the help of a vacuum cleaner, was passed twice through a sieve, disseminated on Petri dishes, and sterilized with dry heat at 160° for 2 hours. The sterile dust was sown with a culture suspension of staphylococcus or streptococcus with a density of 20--30 billion microbial bodies in 1 ml. Subsequently the seeded dust was treated by the method described by V. V. Vlodavets (1960). The dust-borne phase of the aerosol was created in the above mentioned chamber by means of spraying dust, sown with the microorganisms being tested, with the help of a powder blower. As a result of the spraying (20--30 mg of contaminated dust) an aerosol was obtained in the chamber which had a particle size within limits from 2 to 50 microns.

The tests were reproduced both in still air and also in an air medium which was found in constant motion. In the latter case for sustaining the movement of the air a two-blade blower was mounted on the bottom of the chamber. It operated at a rate of 40 rpm.

In various periods of time after sowing of the air medium of the chamber samples of air were taken by the sedimentation (cup) and aspiration (Rechmenskiy bacteria trap) methods. For detection of streptococcus we used Garro medium, and for staphylococcus - salt agar.

For collection of air samples by the sedimentation method two cups with the corresponding selective medium were exposed in the chamber (5 minutes each time). After this exposure the dishes were removed from the chamber and incubated for 24 hours at 37°. The number of colonies from a series of these dishes, successively exposed in the chamber up to 24 hours from the onset of seeding of the air,

made it possible to determine the intensity of sedimentation of particles from the aerosol of the microbe being tested.

The number of viable microbes which remained suspended in the air was determined by the aspiration method. For this purpose 10 liters of air was passed through the Rechmenskiy bacteria trap after the presence of the corresponding microorganism in the air was no longer recorded by the cup method. The liquid from the bacteria trap was inoculated on the same selective media. The seedings were incubated for 24 hours at 37°. Based on the number of colonies which grew on the dishes with the selective media, recalculated for the volume of air passed, we established the number of viable specimens which remained in a suspended state in the air medium of the chamber.

A series of tests was set up for studying the survival of staphylococcus and streptococcus, suspended in the air in the droplet phase of an aerosol, depending on the temperature of the surrounding medium. As the investigations showed, the most stable were aerosols which were formed on distilled water with 1% serum.

The results of these tests by individual groups are presented in figures 1 and 2. As can be seen from the data given in the drawings, with an increase of air temperature there is a regular decrease in the number of both streptococci and staphylococci which are isolated from the aerosol, i.e., under these conditions their death is speeded up.

Thus, at a temperature of 22-25° in 6 hours after spraying of a suspension of staphylococci 28.7% microbes was revealed, while at a temperature of 14-17° in this same interval 45.3% remained viable. In 12 hours 22% of the staphylococci remained viable in the first case, while in the second case 32.5% of the microbes remained viable in the air. And, finally, after 4 days at a temperature of 22-25° 0.72% of the staphylococci remained viable, and at a temperature of 14-16° they were isolated in the proportion of 8.2%. It is necessary to note that in the last case (at 14-16°) staphylococci were isolated (0.5%) from the air still on the 8th day after creation of the aerosol.

An analogous regularity is observed in the series of tests relative to the survival of hemolytic streptococci in the droplet phase of an aerosol under the same conditions. At a temperature of 21-25° quite rapid death of streptococci is recorded. Thus, in 6 hours after spraying of a suspension of this microbe 3.5% of streptococci is isolated from the air (96.5% perished). At a temperature of 14-16° in this same interval 18% of the streptococci remained viable. In the course of 72 hours the air of the chamber was already practically sterile, since in samples of test and control investigations streptococci were not detected.

In comparing the results of these tests it is possible to note that streptococci are less stable in air than staphylococci.

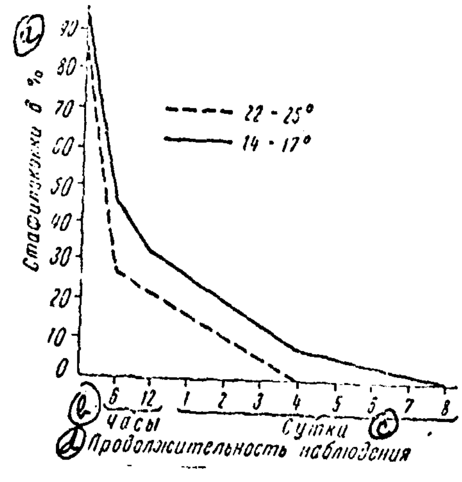

Figure 1. Survival of staphylococci in the air of a chamber at various temperatures.
Key: (a) Staphylococci in %; (b) hours; (c) days; (d) Duration of observation.

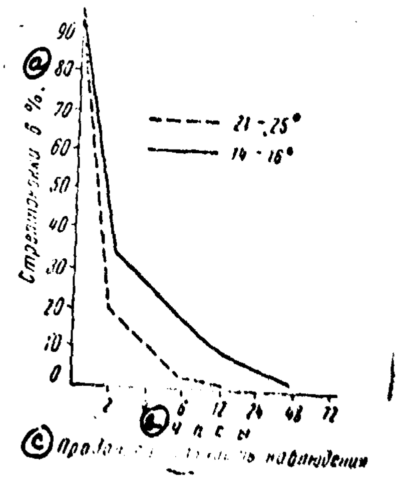

Figure 2. Survival of streptococci in the air of a chamber at various temperatures.
Key: (a) Streptococci in %; (b) hours; (c) Duration of observation.

Table 1

Influence of humidity on the survival of streptococci and staphylococci in the air

a Группа опытов	b Число опытов	c Наименование микроба	d Температура (в градусах)	Сухой воздух (контроль) e					Влажный воздух (опыт) f					i Отношение жизнеспособных микробов во влажном воздухе (%) к сухому через			
				g относительная влажность (%)	h количество микробов после распыления суспензии через				g относительная влажность (%)	h количество микробов после распыления суспензии через							
					30 минут j	60 минут k	2 часа l	4 часа m		30 минут j	60 минут k	2 часа l	4 часа m	30 минут j	60 минут k	2 часа l	4 часа m
n Первая	7	r Стафилококк гемолитический	16—17	42—57	4760	2640	1520	792	84—92	6340	3880	2160	1030	133	145	142	131
o Вторая	9	r Стафилококк гемолитический	27—29	45—60	810	527	382	214	85—92	814	506	391	220	100	100	100	100
p Третья	5	s Стрептококк гемолитический	15—16	40—55	2360	1510	670	316	82—88	3020	1680	483	177	126	111	72	56
q Четвертая	7	s Стрептококк гемолитический	26—28	42—57	247	102	56	28	80—90	239	111	52	25	100	100	100	100

Key: (a) Group of tests; (b) No. of tests; (c) Name of microbe; (d) Temperature (in degrees); (e) Dry air (control); (f) Moist air (test); (g) Relative humidity (in %); (h) Number of microbes after spraying of a suspension in; (i) Ratio of viable microbes in moist air (%) to dry in; (j) 30 min; (k) 60 min; (l) 2 hours; (m) 4 hours; (n) First; (o) Second; (p) Third; (q) Fourth; (r) Hemolytic staphylococcus; (s) Hemolytic streptococcus.

In these series of tests the humidity of the air varied insignificantly (fluctuations were recorded with limits from 40-55% to 43-59%). Therefore it can be considered that here the tested microorganisms died mainly due to the temperature influence, since the latter changed within considerable limits (from 14 to 25°). Apparently under these test conditions humidity did not play a significant role.

For clearing up the influence of humidity on the survival of streptococci and staphylococci which are suspended in the air a special series of tests was set up in two variants. In the first variant the investigations were conducted at a comparatively low temperature (16-17°); in the second variant, conversely, at a higher (27-29°) temperature. In both variants a comparison was made of the influence on these microorganisms of a relative air humidity within limits of 40-57 and 84-92%. The increase of relative humidity of the air was achieved by wetting it with water vapor.

The results of the investigations by groups of tests in this series are given in Table 1. As can be seen from the data in Table 1, at a temperature of 16-17° and increased relative air humidity there is an increase in the survival rate of staphylococci which are suspended in it. Thus, in 30 minutes after spraying of a suspension of staphylococci the number of microbes isolated from moist air (test) comprised 133% relative to the number of microbes detected in dry air (control); in 60 minutes the number of viable microbes in the test investigations comprised 145%, in 2 hours - 142%, and in 4 hours - 131%. It follows from this that at a temperature of 16-17° for the entire extent of the test (duration of 4 hours) in moist air more staphylococci were revealed in a comparison with dry air. Consequently, an increase of relative humidity at a comparably low temperature under conditions of an enclosure (16-17°) promotes the more prolonged survival of staphylococci.

In the third group of tests, conducted with an aerosol of streptococci at the same temperature (15-16°), in 30 and 60 minutes after spraying of a suspension of microbes in moist air a greater number of microbes was detected (correspondingly 126 and 11%) than in dry air, just as in the previous group of tests. Subsequently, however, an opposite phenomenon is observed, i.e., a decrease in the number of streptococci under conditions of moist air (test) in comparison with non-moist (control). Thus, in 2 hours the number of streptococci detected at increased humidity comprised 72%, and in 4 hours - 56% relative to the number of them in dry air. Thus an increase of relative humidity has a positive influence on the stability of streptococci in the air only in the first 60 minutes after they have been sprayed. Later, conversely, in moistened air the intensified death of streptococci is recorded in comparison with dry air.

In the second and fourth groups of tests, conducted at a temperature of 27-29°, an increase of relative humidity did not exert a favorable influence on the stability of these microorganisms in air. Under these conditions both at a low (40-60%) and high (80-92%) humidity the quite rapid death of streptococci and staphylococci is noted. Here it is necessary to stress that under the conditions created for the entire period of the test streptococci were separated from the air in a lesser quantity than staphylococci. Consequently streptococci in an aerosol state are more sensitive to unfavorable factors of the external environment in comparison with staphylococci.

Investigations for studying the aerodynamic properties of the dust-borne phase of an aerosol of the tested microorganisms were carried out at a temperature of 18-22° and relative air humidity of 45-60%. One hour after the seeding 7.5% of streptococci and 17.5% of staphylococci remained viable in it. Two hours after formation of the dust-borne phase of the aerosol 4% of streptococci and 12.5% of staphylococci preserved their viability.

By the end of the 8th hour of existence of the aerosol 6% of the staphylococci and 0.1% of the streptococci remained viable in the air. These findings also testify that staphylococci in the dust-borne phase of an aerosol are more stable than streptococci.

Also important are findings on the length of survival of staphylococci and streptococci on various objects in the environment surrounding man following their contamination by droplet and dust-borne phases of aerosols. For clearing up this problem various test objects - fragments of cotton, wool tricot fabric, glass plate, porcelain, and wood - were placed in an hermetic chamber and seeded with the test microorganisms in the droplet or dust-borne phase of an aerosol.

The microbe-infected test objects were transferred to glass desiccators, the covers of which were equipped with a cotton filter (for ensuring the entry of sterile air), and preserved under various conditions of accommodation for a prolonged period. During storage the test objects were subjected to bacteriological investigation.

Colonies of staphylococci and streptococci, sown out from the test objects after various periods of storage, were subjected to a thorough study. Here their morphological and biological properties were checked, which made it possible to obtain a concept not only of the duration of survival, but also of the preservation of biological activity by these microorganisms.

The duration of preservation of viability and biological activity of staphylococci and streptococci on the tested objects, as the observations showed, fluctuated from several days to several months. The duration of survival of these microorganisms under various conditions of storage of the test objects is influenced by the

initial amount of them applied to this or that object. In a small quantity (tens of cells per 1 cm^2) survival is preserved for from 2-15 days (streptococci) to 6-27 days (staphylococci); in a large quantity (tens of thousands) the microbes survived, as a rule, for from 3-5 weeks (streptococci) to 3-8 months (staphylococci). On objects which were stored in the dark these microorganisms survive 2-3 times longer than under conditions of natural light.

Biological activity (virulence) of the tested microorganisms under those conditions is also preserved for different periods. Thus, staphylococci on objects which were sown with the droplet phase of the aerosol under conditions of darkness preserved virulence for white mice for from 6-16 to 50-84 days. With the admission of natural light they lost their virulence correspondingly in from 5-8 to 18-45 days. Virulence of staphylococci, sown on objects in the form of the dust-borne phase of an aerosol is preserved for more prolonged periods. Under conditions of natural light in the bacterial dust these microorganisms remained virulent for from 9-17 to 54-95 days. Staphylococci preserved their virulence for the longest period in the dark (correspondingly from 20-36 to 142-176 days).

An analogous regularity is noted also in respect to streptococci. With the admission of natural light they lost their biological activity comparatively rapidly - in the droplet phase by the 3-7th, and in the dust-borne phase by the 8-18th day. In the dark these properties of streptococci were preserved correspondingly from 6-14 to 20-25 days.

These findings testify that hemolytic staphylococci, sown on various objects, are little sensitive to desiccation and therefore are preserved on them for long periods both in the dark and with the admission of scattered light. Hemolytic streptococci turned out to be quite sensitive to desiccation under the same conditions of their existence. Thus, hemolytic staphylococci, being preserved on seeded objects under unfavorable conditions for longer periods in comparison with hemolytic streptococci, without a doubt present a greater threat both in a sanitary and epidemiological respect.

Conclusions

1. The periods of preservation of viability of hemolytic staphylococci and streptococci in an aerosol state depend not only on the temperature-humidity regimen of the air medium, but also on the nature of the liquid used for preparation of suspensions of them. Those microbes preserve viability for the longest period in an aerosol which is formed from a suspension prepared on distilled water with 5% serum.

2. Hemolytic staphylococci in an aerosol, formed on distilled water with 5% serum, remain viable for 8 days, while hemolytic streptococci under these same conditions are revealed in the air only for 3 days. Biological activity of streptococci here is preserved for 1 day, and of staphylococci - for 6 days.

3. Staphylococci, found in the form of the droplet or dust-borne phase of an aerosol, are little sensitive to the influence of room temperature (16-24°) and moderate relative humidity (45-60%). At a high relative humidity (80-87%) and increased temperature (27-28°) their necrosis in the air is speeded up noticeably. Under these conditions they are preserved in the aerosol state for 3 days.

4. Streptococci in an aerosol state are very sensitive to increased temperature at harmfull indices of relative humidity (45-60%). High relative humidity (80-87%) with increased temperature (27-28°) intensifies the influence of the latter on their survival in the air. Under those conditions streptococci are preserved in an aerosol for only 12 hours.

5. Maximum periods of survival of staphylococci (8 days) and streptococci (3 days) in the air and the preservation of their biological active (correspondingly 6 days and 24 hours) are recorded at a temperature of 14-16° and moderate indices of relative humidity (45-60%). Apparently an increase of relative humidity at a comparatively low temperature of the air medium of the accommodation can promote the effect of aerogenic contamination.

6. On objects, sown with the droplet phase of an aerosol, staphylococci in the dark at room temperature remain viable for from 13 to 106 days. Their virulence under these conditions is preserved from 6 to 84 days. With the admission of natural light they are preserved for from 5 to 64 days. Here the staphylococci lose their virulence in 3-45 days.

7. Staphylococci, seeded on objects in the form of the dust-borne phase of an aerosol, under conditions of darkness survive for a longer period (from 37 to 212 days). Their virulence under these conditions is preserved for from 20 to 176 days. With the admission of natural light they remain viable for 18-115 days, and their virulence is preserved for 9-95 days.

8. Streptococci, seeded in the form of the droplet phase of an aerosol on objects, are preserved in the dark for 4-12 days; their virulence is lost in 2-6 days. Upon admission of natural light they remain viable for 2-8 days, and they lose their virulence in 1-4 days.

9. Streptococci, seeded on objects in the form of the dust-borne phase of an aerosol, preserve their viability in the dark for from 10 to 62 days. Their virulence is preserved for from 6 to 37 days. With the admission of natural light their viability is reduced to 6-35 days, and virulence is preserved for from 3 to 20 days.

10. Hemolytic staphylococci are preserved in an aerosol state and on objects of the work unit for considerably longer periods than hemolytic streptococci. Therefore, in the capacity of sanitary-significative microorganisms for an appraisal of the air medium of sick quarters it is necessary to consider not streptococci but hemolytic staphylococci.